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RESEARCH IN THE INFECTIOUSNESS OF VIRAL NUCLEIC ACIDS

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One of the most characteristic properties of viruses is infectiousness. It has served, among other things, right from the very beginnings of inframicrobiology, as the characteristic criterion of strains isolated from nature or maintained in the laboratory. Infectiousness, in its turn, is the exteriorization of the rate of multiplication of the noxiousness and the virulence of the elementary corpuscles introduced into the cells.

The phenomenon of multiplication of viruses, a phenomenon realized exclusively in the interior of the host cell, has occupied the attention of scientists during the course of the years, starting from the first experimental innoculation of a virus and continuing on to the present time. Many hypotheses have been submitted during these years to explain the intimate substratum of the mechanism of infectiousness and the multiplication of viruses. We intend to point out that the term "multiplies" as applied to viruses does not match the current basic concepts derived from experimentation; we intend to replace this term with "are multiplied," accenting through this the basically active role of the cell and not of the virus. Indeed, today we know that the virus or only its nucleic acid penetrates into the cell and causes the metabolic laboratory of the cell to synthesize a nucleic acid similar to the viral acid. On these new molecules, the cell also groups the amino acids necessary for the building of protein molecules. These are specific and characteristic of the new virus, but nevertheless identical from all points of view tith those of the virus which infected the cell. Research carried out in the last few

years has proven that the active, infecting, inductive portion of a virus is concentrated in its nucleic, ribonucleic or disoxyribonucleic acid. When this acid penetrates into the interior of the cell, it induces the synthesis of a nucleic acid similar in nature, the skeleton of the complete virus.

The first inframicrobe, for which it was proven that it was not necessary for it to penetrate completely into the interior of the cell in order to cause its synthesis, was the bacteriophage. It has been demonstrated that it is sufficient for the substance in the interior of the bacteriophage (30), composed of disoxyribonucleic acid, to be introduced into the body of the bacteria, since, after about 20-30 minutes it assists in the exploding of the bacteria and the giving off of an immense quantity of bacteriophagic corpuscles identical to the initial ones. This discovery has been verified both morphologically and with the aid of the electronic microscope (46)(7), which shows the bacteriophages on the surface of the bacteria, flattened, without a content which would normally sustain them chemically or architectonically.

Thus, in the stage after the infecting, called the period of eclipse, in the absence of any morphological traces of the presence of the phages in the bacteria, chemistry shows that after the entrance of the disoxyribonucleic acid (DNA) into the bacteria, the latter slowly synthesizes the DNA and even the ribonucleic acid (RNA) itself. The metabolism of the bacteria is deviated from the rapid synthesis of the nucleic acid of the invader phage (15, 36, 37). This aberrant synthesis takes place together with the explosion of the cell and the giving off of new corpuscles, bacteriophages, complet· from all points of view.

The experiences of Hart (29) brought new data in respect to the role of viral nucleic acid. This author found that the degradation with the aid of RNA of a terminal fragment of a maximum of 5% of the entire RNA acid from the virus of the mosaic of tobacco is accompanied by a total loss of infectiousness.

These studies, the first of their kind, opened new horizons in regard to the role played by the nucleic acids in the composition of viruses.

The situation was at this point when, in 1957, a number of precise and correctly managed tests produced very important data concerning the infectiousness of viral ribonucleic acids. In a few years, the results accumulated by numerous laboratories, grew to be impressive, both through their value and their contribution.

Owing to the limited space, we will be able only to sketch some of these results and we shall emphasize greatly the studies made in our laboratory.

In 1956, Gierer and Schramm (27) obtained the infecting ribonucleic acid of the tobacco mosaic virus through phenol extraction. At the same time, Fraenkel-Conrat (20) degraded the protein of the same virus with a detergent and liberated the same infecting nucleic acid. If the RNA solution extracted from the virus with one of the techniques is introduced into healthy leaves, they become sick and show all of the signs characteristic of the mosiac disease. The diseased leaves contain an immense number of virotic corpuscles, identical from all points of view to those from which the RNA was extracted.

These studies demonstrate the active, infecting role of the compound extracted from the virus and its inductive character in relation to the sensitive cell. It was thus proven that it was not necessary for the entire virus to penetrate into the cell in order to infect it; this role is possessed only by ribonucleic acid.

After this first step on a new road, a step which permitted the conception of new ideas, there was an avalanche of works during the following years which showed that not only the virus of the tobacco mosaic disease contains a ribonucleic acid with an inductive character, but also numerous other viruses. The list of these is long and we shall list only a few of them: Mengo, West-Nil, type 2 poliomyelitic (16, 17), equine encephalomyelitic (99), type 1 and 2 poliomyelitic in tissue cultures (3) (4), murine encephalomiocardiac (34, 35), aphthous (11, 12, 97), Semliki-Forst (14), the virus of the ring-shaped petals of tobacco (39), Coxsackie (40), enteroviruses (96), the virus of the ring-shaped petals of cucumbers (19), Murray-Valley encephalitis (8), the bushy Stunt virus of tomatoes (91), the Group B arboviruses (49), etc. Of simple, nucleoproteic composition, these viruses give off an infection-causing ribonucleic acid through chemical treatment which causes the sensitive cell to synthesize a complete virus, similar to that from which the active compound was extracted. The logical conclusion suggested to us in the results recorded with the ribonucleic acid of the tobacco mosaic disease virus remains valid also for these viruses: the infectiousness of the virus is ensured by its nucleic acid; the multiplication of the elementary corpuscles is not realized by binary division; the sensitive cell synthesizes the complete virus in its entirety, starting with a simple and unique model -- the molecule of viral ribonucleic acid.

In 1957, we carried out our first tests to prove that the viruses which were chemically and morphologically complex were also infectious through their nucleic acid. The first work in a series of studies was published in 1958 (51). On this occasion, we proved that the ribonucleic acid extracted from influenza virus, a large and complex-structured virus, can induce the synthesis of the initial virus through its presence in the cell.

We later proved that another mixed virus, the Newcastle virus, gives off an active ribonucleic acid (52) under the combined action of ether and phenol.

Chemical analysis has shown that the influenza virus contains nucleic acid (NA) in a much lower proportion (around 0.8% (1), (24)) than the viruses from which an infectious RNA (5% in the virus of the tobacco mosaic disease (44) and 30% in the poliomyelitic virus (93)) had been extracted. It was also proven that the influenza virus also contains proteins (24, 43), lipides (24), (98), (23), glucides (23), (23, 22), water (45), and a hemoglutine of an enzymatic character, which gives it some special properties (24, 13). Recent studies with an electronic microscope and using a technique of special coloring have brought out the inner structure of the influenza virus. On this occasion, it was found that each elementary corpuscle had short, thread-shaped extensions on its surface; these many extensions were interpreted by the authors as hemagglutinin molecules (32). In the interior of the corpuscle there was found a long, covered filament composed of specific viral nucleoprotein. This can be extracted and displayed under optimum conditions after destruction of the covering membrane with the aid of ether (33).

Our studies have pinpointed the nature and character of the ribonucleic acid extracted from the influenza virus, as well as that of the virus synthesized under the action of this compound.

The infectious ribonucleic acid was extracted with the aid of a saturated (over 80%) solution of phenol, after delipidizing the virus with ether, a technique developed by us (51). The RNA solution obtained was immediately innoculated into the alantoidian cavity of an embryonic chicken egg in variable quantities, depending on the virus strain used, the concentration of the virus in the alantoidian liquid of the initial culture, and the RNA concentration in the extract obtained. The innoculation of the RNA into the alantoidian cavity brought about an abundant synthesis of influenza virus, confirmed through the hemagglutinin titres; these titres increased on even the first

passage and continued to increase in later titres (51). It should be noted that sometimes a number of eggs innoculated with RNA give off an alantoidian liquid that lacks hemagglutinin properties; however, after innoculation of another egg there is an abundant culture of influenza virus and high hemagglutinin titres (59). These results are observed especially when the innoculation dose of RNA is at the lower limit of its infectiousness and owing to this fact generates a lesser number of corpuscles, a quantity insufficient to determine the hemagglutinin phenomenon, but sufficient to determine in the next passage in the egg embryo the appearance of an abundant culture of influenza virus and, consecitively, of hemagglutinination. The strains of influenza virus which most constantly have given an infectious product have belonged to type A (54). The specificity of the original virus was preserved in the RNA molecules which transmitted the new virus to it. We observed, nevertheless, one exception when we used a strain of influenza virus with particular antigenic properties. This was the case of the antigenic, type A virus, which has a secondary fraction of type A_1, whose nucleic acid causes the synthesis of a strain of pure type A (53).

In our studies we noted a certain correlation between the seasons and some negative results in attempts to reproduce the influenza virus. Actually, we were able to observe how these failures took place with a certain periodicity, that is, they coincided with the season in which the tests were made, i.e., in the fall and winter months (65, 66).

The RNA extracts obtained from the influenza virus were subjected to control tests. Thus, after 3 hours at 37 degrees, our extracts became completely inactive, while the influenza virus remained active for at least 3½ hours under the same conditions. Kept for 24 hours at laboratory temperatures, our extracts became inactive, while the suspensions of influenza virus remained active. Finally, our RNA solutions were completely inactive in the presence of ribonuclease, after a contact of 20 minutes at 37 degrees, which was not true for the corpuscular influenza virus (55). The results of these tests indicate that the solutions of RNA extracted from influenza virus no longer contain viruses in their corpuscular form, since the entire infectious activity of these solutions is exercised by the molecules of nucleic acid. In order to show the possible proteins in our extracts in the form of intact molecules which might still exist, we subjected the RNA solutions to a chromotagraphic analysis on paper, a method which we had found to be more exact and more sensitive than the reactions currently used. The chromatograms were realized with dialysed extracts so as to eliminate the amino acids resulting in the course of the extraction (61, 65). They proved the absence of intact

proteins in the phenol extracts; the chromtaography of the hydrolized acid of the RNA solution, in contrast, made it possible to bring out under ultraviolet light the presence of the four bases of ribonucleic acid -- guanine, adenine, citozine, and uracil -- which constitutes a positive identification of much greater value of the nucleic acid type (85).

Spectrum analysis of infectious RNA extracted from influenza virus showed us under ultraviolet light an absorption maximum at 258 mμ and the absence of the characteristic maximum of proteic compounds (68).

The morphological aspect of the influenza virus reproduced by the RNA, as well as the nucleic acid, were the object of several studies using the electronic microscope (60, 69). The innoculation of RNA into the embryo chicken egg induced in the cells of the coriolantoidian membrane the generation of some influenza corpuscles, the form of which was spherical and fibreous. The size of the spherical formations is an average of 103.4 mμ, therefore slightly more than that of the original strains, for which we found an average of 101 mμ (56). It must be noted that in this first culture we did not encounter the flattened forms, which would represent, in our opinion, the incomplete elements present in the initial strain. The corpuscles generated after the first passage in the embryonic egg of this virus have different morphological and size characteristics, both for the virus of the initial strain and for that induced by RNA. Thus, in addition to the spherical forms, some cylindrical aspects were also observed. The size of the majority of the spherical forms is 99.5 mμ, although some measure 113.7 mμ and represent flattened forms which we mentioned above. The general aspect of the curve of the dimensions of this virus resembles, significantly, the curve of the initial strains of virus which served for extraction (60).

The presence of a multitude of fibreous forms between the spherical corpuscles generated after innoculation of the RNA and the lower infectiousness recorded in tests to transmit experimental infection with RNA through the nasal passage to mice (51), both of which are specific characteristics of the type A strains recently isolated in an egg, suggest to us that under our conditions the RNA has characteristics similar to those of the influenza virus strain recently isolated from nature (65).

Our results were confirmed after less than one year by Maassab (47), who also found RNA infectiousness in RNA extracted with cold phenol from the coriolantoidian membrane (CAM) of a chicken egg embryo or from cell cultures of chicken kidneys infected with the asiatic flue virus strain.

In recent years, Isaev and Jumatov (38) and then Poliac and Dubrovina (88) have succeeded in extracting an infectious RNA, also from the type A influenza virus, for cell cultures, the chicken embryo and white mice.

A modification in the extraction techniques which we developed, through the introduction of preliminary removal of the lipides in the virus with ether, has led to good results in both the experiments of Maassab (48) and of Sokol and Schramek (95), as well as other scientists (38, 88).

Authors who have studied the activity of RNA extracted from influenza virus (88, 48) have not given sufficient emphasis to the inconstancy of positive results achieved when they sought the infectiousness property; this is a fact pointed out for the first time in our work. The number of negative results varies, according to authors, from 20% up to 70%. In the tests made by us, the percentage of negative results listed was about 40%. Numerous tests undertaken to explain the causes of these failures have not succeeded in completely explaining this phenomenon.

The extraction of an infectious RNA from influenza virus depends on, upon other things, the virulence of the strain, which, in turn, is connected with the number of passages made in the laboratory after isolation, as well as the type of strain. In our very first tests we noted that the strains of influenza virus that had been passed through the chicken embryo a number of times gave inconstant results, while the recently isolated strains gave off a much more active RNA (54). Also, the strains belonging to type A_2 never gave off an active product (55). Studies made with the electronic microscope made it possible in this latter case for us to reach the conclusion that the type A_2 strain with which we were working contained over 50% flattened corpuscles, interpreted as incomplete (57, 58). It is known that these viral elements are poor in RNA, as can be proven by the chemical analyses made by Ada (2).

On the other hand, Maassab (48), in his experiments, did not succeed with any method in extracting an infectious RNA from porcine influenza nor from strains PM 1 and PM 8 of influenza virus.

Other causal factors of the experimental failures are: the method of extraction, which, without lipide removal, produced a larger number of negative results; the quality of the substances used (67); the low titre of the virus from which the extraction was made; the ionic power of the solution in which the dilution was made (38); the sensitivity of the tissue into which the RNA was injected (51, 47) etc.

The scientists who extracted the infectious RNA from influenza virus have emphasized the fact pointed out by us in advance and mentioned above, that is, that morphologically and antigenetically the virus generated from RNA is identical to the virus used for extraction (51, 47, 88, 48).

The results of the studies on influenza virus have made it possible for us to reach some synthesized conclusions, as follows: ribonucleic acid is the factor which causes the infectiousness of the virotic particle, that is, after the disintegration of the particle, the ribonucleic acid, without the lipide, glucide and protein fractions, causes experimental infection. These results support the point of view of synthesis of the virus in toto by the cell, starting from the innoculated molecules of ribonucleic acid which serve as the model. We are inclined to believe that the cells, in accordance with this model, synthesize the identical molecules of virotic nucleic acid, since the small quantity of nucleic acid from the innoculation can not justify the immense number of viral corpuscles freed by the cell. The new nucleic acid, which is endogenous and fabricated by the cell under the influence and according to the type of exogenous nucleic acid introduced into the cell, serves as the skeleton on which the cell continues to deposit the other constituents of the virus, respecting and reproducing the specificity of the initial virus. The virus thus synthesized possesses, when it leaves the cell which generated it, the totality of the characteristics of the influenza virus.

Continuing on this line of study, we tried also to prove that the disoxyribonucleic acid of viral origin also had infectious properties. For these studies, we used type 3 adenovirus. We used a strain which had been kept in the laboratory through successive passes through human amniotic cell cultures in a continuous line (70, 71) and a strain kept in successive passes through human embryo cells cultivated in vitro after trypsinization (74). The phenol extraction, performed according to our technique, permitted us to obtain a nucleic acid with infectious and inductive properties in relation to the amniotic and embryonic cells cultivated in vitro. Chemical and spectroscopic study of this compound showed a pure disoxyribonucleic acid which had no undergraded viral corpuscles or protein corpuscles (71). The product is constantly infectious, sensitive to the action of disoxyribonuclease, and insensitive to the action of ribonuclease (74). The fact must be mentioned that the DNA extracted from the strain of adenovirus maintained in amniotic cells generates the appearance of the cytopathic affect (CPE) in a period of time much shorter after innoculation of the amniotic cells than when the human embryo cells are innoculated (71). This fact appears to be connected to the cell type in which the virus strain used was kept in the usual

manner. An argument in favor of this point of view is that the virus, once synthesized under the influence of the DNA, shows, in relation to the cell into which it is later injected, identical properties to those of the initial virus, at least in respect to the incubation period and the intensity of the lesions observed. In connection with this, we mention that the hypothesis made by one of us (63), that a virus placed in contact with a new tissue system does not succeed in inducing through its nucleic acid its synthesis with the maximum efficiency at the prime moment, owing to the incapacity of the cell to synthesize a nucleic acid that is very different from that which it normally prepares.

In the case of the strains maintained in embryonic cells, the DNA has a shorter incubation period than the DNA extracted from the strain maintained in amniotic cells, and even the virus which was used for extraction (74). This fact is explained by the use, in the extraction of DNA from the adenovirus maintained in the embryonic cell, of a solution of 2 M NaCl instead of 0.14 M NaCl. The use of solution with increased molarity allows, as was shown by Alexander and collaborators (41) and Boeye and collaborators (9), a greater permeability of the cellular membrane in respect to the molecules of nucleic acid. On the other hand, from observations made of these cultures, it appeared that the short duration contact with a solution of increased molarity had as an effect a stimulation of the cellular metabolism, which was translated morphologically by an intense multiplication of the cells in the culture and was reflected, in the final analysis, in the multiplication of the virus. The increased molarity of the solution used for extraction also had the purpose of slowing the DNA precipitation and, therefore, the obtaining of larger concentrates of nucleic acid.

This could be the mechanism which is the basis for the explanation of the shortened incubation period. Added to this is the importance of the cell type in which the virus strain is maintained for extraction. An argument in favor of this is the fact that in the last passing through the incubation period increases and very quickly becomes equal to that of the whole virus. We mention that during these passings the molarity of the solutions is physiological and, therefore, the cells also present a normal reaction.

The seroneutralization reactions made between the virus strain generated by DNA and the anti-adenovirotic serum of type 3 shows us that the virus generated is actually an adenovirus of the same antigenic type as the initial virus, i.e., type 3 (74).

In other experiments, we were able to prove that two strains obtained from the cultivation of type 3 adenovirus in Ehrlich ascitic carcinoma can, under the action of cold phenol, give off DNA which generates in the human embryo cells the synthesis of viruses with infectious and cytopathic properties.

We emphasize that in these latter experiments the strains generated from nucleic acids have modified characters, i.e., characteristics modified in the same antigentic sense as the viral strains cultivated in vitro in the Ehrlich ascites. This fact seems of great importance to us since it allows for the possibility on the one hand of intermolecular hybridization in vivo and on the other hand of the transmission only through the DNA of the newly acquired characteristics.

Indeed, the preservation of the new characteristics by the virus generated after innoculation with DNA extracted from the new virus strains in cultures of human embryo cells represents proof of the fact that the process of transformation suffered by the type 3 adenovirus was realized at the level of its nucleic acid. At the same time, the transmission of these characteristics only through the virotic DNA demonstrates to us on the one hand, even more, that the specificity of the virus is due to its nucleic acid, and on the other hand that the changes in structure which took place are stable enough in order to be transmitted only by the DNA.

The experimental results realized in our tests in respect to the infectious role of the nucleic acids verify the hypothesis previously made (63), which authorizes us to say that "for the first time they have provided proof that the viruses which are large and chemically complex have as a unique factor, in the manifestation of at least two of their characteristics, i.e., infectiousness and inducement of intracellular synthesis, a single necessary element, nucleic acid, either in the form of ribonucleic acid or in the form of disoxyribonucleic acid" (65).

In continuing our studies, we also treated, among other things, the problem of the inactivation and reactivation of the viral nucleic acids. The study of the action of some chemical and physical agents on the infectiousness of nucleic acids (72) showed us, among other things, that the inactivation mechanism is not due to an oxidation process as claimed by other authors (25). On the other hand, the reduced substances do not slow up the loss in the activity of the extracts. At this time, we developed, for the first time, the hypothesis in accordance with which the loss in activity was due to the breaking off of

intramolecular bonds as the result of the thermal agitation existing in the solution of nucleic acid (72).

Other studies permitted us to determine one other significant fact not given in literature: RNA extracted from MM virus and heated at 60 or 75 degrees loses its infectiousness after 20 and 25 minutes respectively and later, continuing the heating, recaptures up to 80% of its infectiousness. We have interpreted these results as caused by the effects of the heat, through the breaking off of some fragments in the active macromolecule with a loss of activity, followed by the later restructuring of the molecules with regained infectiousness. Experiments have shown that the cooling model tests have a primary role in reforming the molecule; this reforming is realized in a greater proportion when the cooling takes place slowly. The importance of the cooling model for retention of the activity of the heated RNA is also emphasized by Goldberger (26). He showed that slow cooling to 37 degrees of the RNA extracted from the Sindbis virus, after heating it to 90 degrees, caused it to have a much lower infectiousness than when it was cooled rapidly to 0 degrees, when the nucleic acid is found in a solution with a concentration of 0.14 M ClNa. In contrast with these results, the cooling model had no influence on the infectiousness of RNA heated to 80 degrees and extracted from the Mahoney strain of the poliomyelitis virus. The author explains these contradictory results by the difference in structure of the two nucleic acids.

The totality of results obtained in this series of studies by us and by other authors make us believe that we can support our point of view, according to which the temporary inactivity of the nucleic acids is due, primarily, to the thermal agitation.

Chromatographic studies (84) and spectrographic studies (79), as well as some of the primary viscosimetric (82) and light diffusion (83) results, have shown us that RNA extracted from the MM virus is formed of two distinct fractions. The first, which we call fraction A, has a low chromatographic migration speed and has a strong absorption under untraviolet light. It represents 90% of the total quantity of RNA. The other, which we have called fraction B, has a greater chromatographic migration speed and a poor absorption speed under ultraviolet light. Both components of infectious RNA, when heated to 75 degrees for a period of time between 5 and 50 minutes and then slowly cooled, show modification in the migration speed and in the extinction values at 260 m . This data, together with other similar results obtained in experiments with tissular RNA (78), reinforces the point of

view mentioned, i.e., that the intramolecular bond breaks and reforms as a result of heating, which explains the mechanism of loss and regaining of virotic RNA infectiousness as a function of heating time.

Chromatographic studies (80) made on a DEAE-cellulose column in continuous flux, using an original technique (81), have demonstrated that the RNA extracted from the influenza virus, strain PR 8, is different from that extracted from CAM. These results form one more proof for the specificity of nucleic acids and for the fact that injection of a virotic RNA into a sensitive cell causes change in the metabolism of the cell and synthesis of virotic nucleic acid.

In light of the results achieved in recent years, it is now necessary to revise some concepts of inframicrobiology which have become outmoded in the current state of our knowledge. Among these is the concept of the infectiousness of viruses; this infectiousness can no longer be connected with the morphological and chemical integrity of the elementary corpuscle, but rather must be restricted exclusively to nucleic acid.

Conclusive proof of the infectious role of nucleic acids is also provided by hybridization experiments realized with the virus of the tobacco mosiac disease (21). It has been proven that in the case when the native protein of an antigenetic type of this virus is linked in vitro with RNA of another type and is injected with the hybrid, the descendants will have all of the characteristics of the virus from whence the RNA came.

The explanation of the multiplication of the viruses as a result of binary division must also be revised. Indeed, the facts listed up to this point indicate that it is not a question of the virus "being multiplied," by binary division or some other method, but rather that it is synthesized by the cell completely. This synthesis is initiated and caused exclusively by the viral nucleic acid, regardless of whether it is ribonucleic acid or disoxyribonucleic acid. We intend to state more precisely that if this mechanism of synthesis is, as we believe, valid for the great majority of inframicrobes, then we are forming again a reserve against pararicketts and ricketts. These have, as we have shown on another occasion (73), a multiplication mechanism which is still inadequately understood and are very different chemically and structurally from other inframicrobes. Confirmation of this point of view seems to be present in some recent experimental results. Thus, Psheniknov and collaborators (89) have succeeded in cultivating two rickettsias in devitalized semi-synthetic media.

Moreover, Crocker and Eastwood (18) obtained multiplication of the ornithosis virus in denucleated, human amniotic cells. On the other hand, Podolitan and collaborators (86), studying the morphogenesis of ornithosis with the aid of cinematography adapted to a phase contrast microscope and using ultrafine sections for the electronic microscope, were able to record on film the aspects of the binary fission of the corpuscles.

In regard to the chemical structure of the viruses which belong to the ricketts and pararicketts group, it has been proven that they are much more complex than the other inframicrobes. Thus, in contrast to the latter, they contain both nucleic acids (90, 100, 87, 94). In addition to this, the rickettsian corpuscles also possess some enzymes, among which are the oxidative and the transaminase (28, 10, 101, 31). Electronic microscopy has shown that the two groups of inframicrobes possess an internal organization similar to that of bacteria, that is, cellular walls, cytoplasma, and differentiated nuclei (5). This chemical, structural and morphological complexity, as well as other special characteristics, cause us again to assert that the microorganisms grouped in the category of rickettsias and pararickettsias have more characteristics in common with bacteria than with inframicrobes.

Immunity and immunization are, as we have pointed out on other occasions, connected with and strictly dependent on the degree of activity and the inductive potential of the nucleic acid derived from the vaccine-virus. Thus, active vaccination, effective in the field of viruses, can be successfully used only when a virus is used which has preserved the infectiousness, for the most part, through a nucleic acid capable of inducing the intracellular synthesis of the homologous virus. A virus in which the nucleic acid has lost the inductive capacity owing to the very strong action of attenuating agents is ineffective (64).

The source of immunity in viruses is therefore cellular, since only inside the cell can the nucleic acid exercise its inductive action which leads to the generation of a more or less complete virus, which, however, can become antigenic.

Adaptability also can no longer be considered as an exclusive property of the inframicrobe, but rather must be looked at as a possibility of the cell to respond more rapidly or more slowly to the incitation toward synthesis of the viral nucleic acid.

The affinity of a virus must be summarized, in the final analysis, as the capacity of the cell host to be able to synthesize or not synthesize infectious nucleic acid.

These are only some of the concepts of inframicrobiology which must, in our opinion, be re-examined through the prism of new data.

Through their nucleic acid viruses also raise a number of extremely interesting theoretical problems in the field of genetics. It is known that the great majority of viruses contain one or ther other of the two nucleic acids. We have seen in the above that the nucleic acid alone can induce in the sensitive cell the synthesis of new molecules of nucleic acid which are identical and then of the corresponding proteins, giving birth to a complete virus which is identical to that from whence it came.

We have observed, therefore, that the nucleic acid (both the RNA and the DNA) has the role of carrier and transmitter of genetic information. In this case, at least two questions are posed. Is it possible that the totality of the character of a virus, speaking genetically, could be transmitted exclusively by one of the two nucleic acids? Experimental studies show, without a shadow of a doubt, starting in 1956 and continuing up to the present, that this is possible in both simple viruses and in complex viruses, in cases involving both types of acids. We must therefore incline ourselves in light of the present experimental argument toward the only valid fact and should recognize that each nucleic acid in turn is the carrier and transmitter of genetic information, in the case of inframicrobes.

The second question is whether the viral nucleic acids can be considered carriers of genetic characteristics in the strictest sense of this concept as we understand it today. In order to answer this, two important facts must not be forgotten. First of all, the crude method of extraction of the nucleic acids, which involves the use of a saturated solution of phenol in the presence of which the virus is maintained for a relatively long period of time. Under these conditions, no micro-organism would be able to preserve the character necessary to pass on hereditary endowments. On the other hand, treatment with phenol destroys the integrity of the inframicrobe morphologically and chemically, leaving unaltered in the solution only the molecules of nucleic acid. Finally, it is well established today that it is no longer necessary to invoke binary division of viruses with consecutive transmission of the hereditary characteristics, since the inductive action of the nucleic acids has been demonstrated. Moreover, the molecules of these compounds penetrating into the cell are not themselves divided, but rather, owing to their presence, the cellular laboratory assumes the task of synthesizing new, identical molecules and, then, the complete virus.

The question that comes up is the following: can the molecules of the viral nucleic acids be considered live molecules endowed with the capacity of transmitting through themselves the whole hereditary baggage of the inframicrobes, or, must they be considered special chemical compounds capable of inducing through themselves the synthesis in only certain cells considered receptors?

As a logical linking in our studies on the infectiousness of viral nucleic acids, we placed for ourselves the working hypothesis of obtaining these active compounds outside of the live cell, using the enzyme method.

The working hypothesis was justified, first of all, by the fact that the molecules of viral nucleic acids must be considered, in our opinion, as chemical molecules in the strictest sense of the word, and therefore not live.

The second argument which justified our studies was the obtaining for the first time of a biosynthesis of nucleic acids by Kornberg (42) for DNA and by Ochoa (50) for RNA. The fact must be mentioned that these nucleic acids were of another nature than the viral and therefore they lacked any property of infectiousness.

The fact that these syntheses are conditioned and directed by the addition of a model strengthened our supposition that the synthesis of viral nucleic acids is also possible.

Using as a synthesis model two nucleic acids -- the disoxyribonucleic acid (77) of the type 3 adenovirus and the ribonucleic acid (76) of the MM virus -- in a medium lacking cells, it is possible to obtain a biosynthesis of both nucleic acids endowed with infectious characteristics. The complex medium employed by us contains, in essence, an acellular homogenate of Ehrlich cells, very metabolized tissue, and an energetic chemical factor, in the ATP species.

The realization of the synthesis in this experimental model or in other experimental models being studied causes us to presuppose that it is possible to obtain in this way a biosynthesis of any infectious nucleic acid of viral origin when it is used as an initiator.

We look on the obtaining of viral, infectious nucleic acids through biosynthesis as a justification and an additional argument for the belief which we have held for many years that the molecules of viral nucleic acids can not be considered live, but rather as chemical molecules endowed with certain

inductive properties which are evidenced when they are introduced into sensitive cells.

We know with certainty today that in a complete virus only the nucleic acid is capable of causing the cell to synthesize this nucleic acid and later the complete virus.

In light of the later data and being obliged to consider an inert and synthesizable m olecule as the sole active element of the virus, we have the right to question once again, but with much greater force, as to whether viruses can be considered as live micro-organisms.

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